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HPLC and FTIR spectral studies of the simple ascidian *Phallusia nigra*

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ABSTRACT

Ascidans are a rich source of bioactive secondary metabolites. Phallusia nigra is a simple ascidian belonging to the family Ascidiidae found in plenty throughout the year along the Tuticorin coast of India. The methanolic extract of Phallusia nigra was subjected to HPLC and FTIR spectral analysis to determine the possible bioactive components. The interpretation of the spectrum showed the presence of aliphatic bromo compounds, phenol or tertiary alcohols, carbonyl compound, carboxylic acids, lipids, proteins, alkanes and aromatic compound.

Key words: *Phallusia nigra*, ascidian, HPLC, FTIR

INTRODUCTION

Ascidians are marine sedentary organisms. *Phallusia nigra* is a simple ascidian belonging to the family Ascidiidae [1]. Hundreds of new compounds have been isolated from ascidians, the majority of which are amino acid derivatives. It is the biological activity associated with many of these natural products that are responsible for research focus on these marine organisms. To date, the most notable examples of bioactive ascidian compounds include didemnin B [2,3], dehydrodidemnin B, ecteinascidin-743 [4,5], sulcatin [6], stolonic acids A&B [7], bistramides A,B,C,D & K [8]. Ascidians are renowned for their overwhelming bias towards the production of nitrogenous secondary metabolites. However, with the continued chemical interest in this group of animals, an increasing number of non-nitrogen containing metabolites are being isolated. Hence the objective of the present investigation is to identify the possible chemical constituents with the aid of HPLC and FTIR spectral analysis.

MATERIALS AND METHODS

Collection of animal material

Phallusia nigra (Family: Ascidiidae) was collected from Tuticorin coast in the month of October 2010 by SCUBA diving. Molluscan shell, calcrete rock fragments attached to the foot of the animal was carefully removed. They were identified using key to identification of Indian ascidians [9]. A voucher specimen AS-2083 has been submitted in the ascidian collection of museum of the Department of Zoology, A. P. C. Mahalaxmi College for women, Tuticorin – 628002, Tamilnadu, India.

Preparation of extract

The whole animal was dried in shade and homogenized to get a coarse powder. The powder was successively extracted with various solvents such as petroleum ether (40^0-60^0 C), benzene, chloroform, methanol and water. The extracts were concentrated in a rotary evaporator under reduced pressure and used for further chemical investigations.

HPLC analysis

HPLC studies were carried out in Shimadzu CLASS-VP V6.14 SP2 system. Column: C 18: Mobile phase Solvent A-Water, Solvent B-Methanol: Pumps (Binary gradient): T.Flow: 1.000 ml/min: P.Max: 400.0 kgf/cm^2: P.Min: 0.0 kgf/cm^2: Temperature: 35^{0}C: Lamp: D2: Polarity: +: Wavelength Ch 1:280 nm: Injection volume: 10 micro liter.

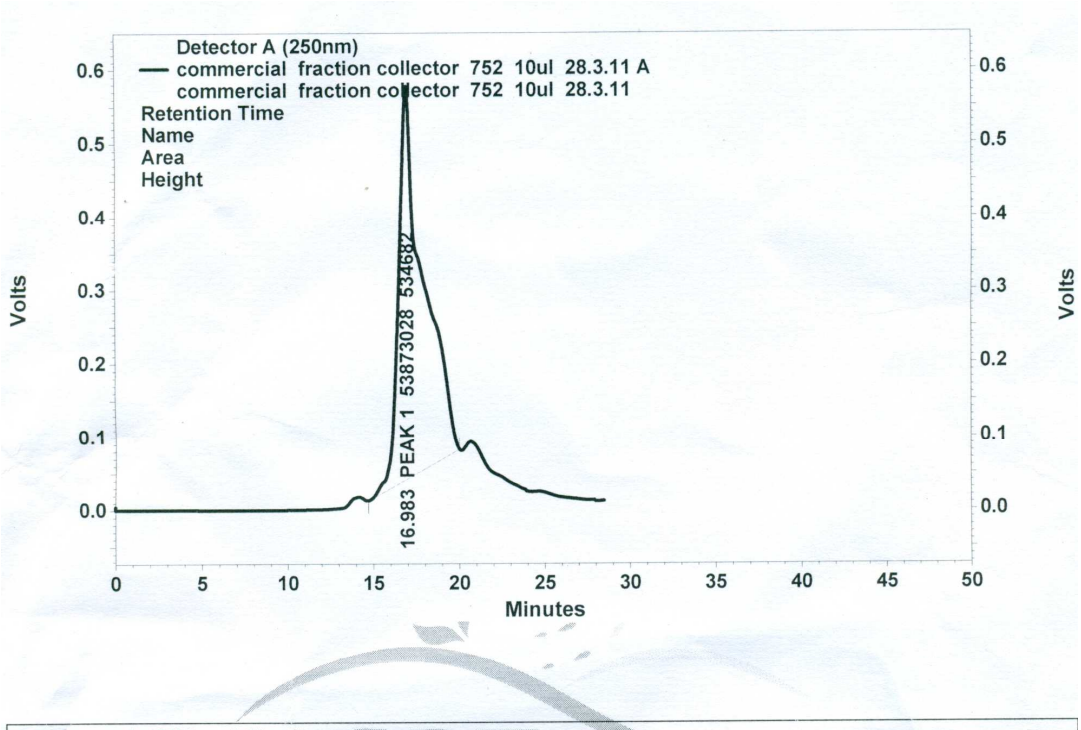
IR spectral studies

Extracts were analyzed in a liquid cell. This is a small container made from NaCl (or other IR-transparent material) which can be filled with liquid, such as the extract for EPA 418.1 analysis. This creates a longer path length for the sample, which leads to increased sensitivity. Sampling methods include making a mull of a powder with a hydrocarbon oil (Nujol) or pyrolyzing insoluble polymers and using the distilled pyrolyzate to cast a film. Materials are placed in an Attenuated Total Reflectance (ATR) cell and gases in gas cells. The following conditions were employed; Perkin Elmer Model spectrum RXI; Range 4000nm-400nm; Resolution 4; Transmittance test mode.

RESULTS AND DISCUSSION

HPLC Analysis

HPLC analysis of the methanol extract of *Phallusia nigra* was carried out to obtain the liquid fraction of maximum quantity. Fig. 1 shows the HPLC chromatogram. The fraction was selected by its maximum peak area and then subjected to FTIR studies.



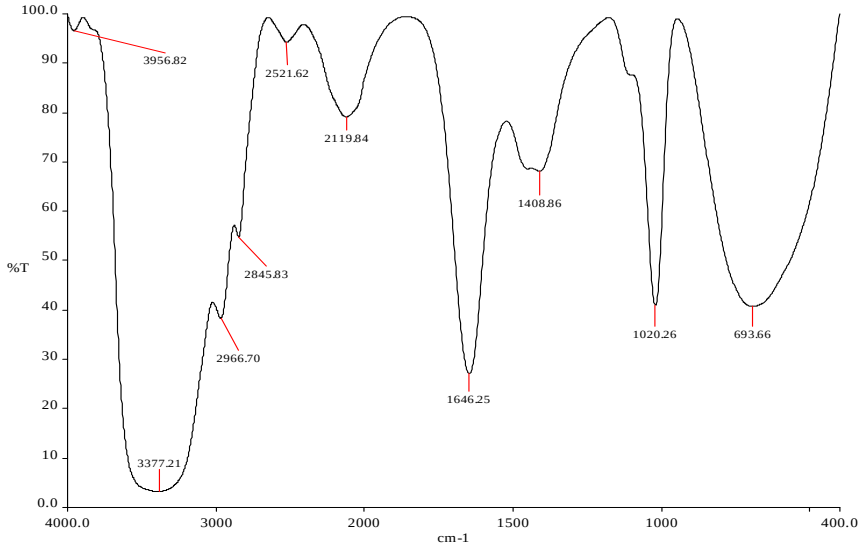
Detector A (280nm)			
Retention Time	Area	Height	Name*
16.983	53873028	534682	PEAK 1

*Parameters tested are not covered under the scope of NABL accreditation

Fig. 1. HPLC Chromatogram of Methanol extract of *Phallusia nigra*

FTIR spectral studies

Fig. 2. shows the FTIR spectrum of selective fraction of HPLC. The spectrum is interpreted and the results are presented in Table 1.



752.pk

752.sp 1801 4000.00 400.00 3.22 99.99 4.00 %T 10 1.00

REF 4000 99.52 2000 86.90 600
3956.82 96.58 3377.21 3.22 2966.70 38.36 2845.83 54.82 2521.62 94.25
2119.84 79.14 1646.25 27.14 1408.86 68.12 1020.26 41.04 693.66 40.71

Fig. 2. FTIR spectrum of selective fraction of HPLC

Interpretation of FTIR results

Table 1: IR spectral data

No	Group Frequency cm^{-1} of the sample compounds	Functional group assignment and compound	Group frequency cm^{-1}
1	693.66	Aliphatic bromo compounds,C-Br stretch	700–600
2	1020.26	Phosphate ion	1100-1000
3	1408.86	O-H bend, Phenol or tertiary alcohol	1410-1310
4	1646.25	C=O stretch, carbonyl compound	1650-1600
5	2119.84	Isothiocyanate (-NCS)	2150–1990
6	2521.62	O-H stretch, Carboxylic acids	3500-2400
7	2845.83	-CH-CH_2 asymmetric stretch- lipids, protein	2865-2845
8	2966.70	C-H stretch, alkanes	3000-2850
9	3377.21	O-H stretch, H bonded-Alcohols, Phenols	3570-3200
10	3956.82	C-H stretch, C=C stretch, Aromatic compound	>3000

Interpretation of Infrared Spectra has been done by the methods suggested by John Coates[168].

The selective HPLC fraction of methanol extract of *Phallusia nigra* with maximum peak area was subjected to FTIR spectral studies. The interpretation of the spectrum showed the presence of aliphatic bromo compounds, phenol or tertiary alcohols, carbonyl compound, carboxylic acids, lipids, proteins, alkanes and aromatic compound.

CONCLUSION

The study clearly indicates that the methanolic extract of *Phallusia nigra* is rich in many bioactive chemical components. However further studies such as isolation, purification and structure determination is required for the development of new drug.

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